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Induced Hearing Loss in Chinchillas

PRINCIPAL INVESTIGATOR: Sandra L. McFadden, Ph.D.

CONTRACTING ORGANIZATION: The State University of New York
at Buffalo
Amherst, New York 14228-2567

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FOREWORD

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Sandra S. McGaugh
PI - Signature

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1. Introduction

Noise-induced hearing loss (NIHL) is a major occupational hazard for military personnel due to the types and levels of noise encountered in training and combat situations (Dancer and Franke, 1986; Henselman et al., 1994, 1995). Damage to the cochlea can be caused by a variety of acoustic events, ranging from prolonged exposure to continuous noises that cause metabolic and biochemical changes in the cochlea, to relatively brief exposures to high-level impact and impulse noises such as gunfire, cannon fire and explosions, that can produce direct mechanical damage as well (Dancer and Frank, 1986; Henderson et al., 1994; Henselman, 1995). A recognition of the serious consequences of NIHL led the U.S. Air Force to develop the first hearing conservation program (HCP) in 1948. The U.S. Navy and U.S. Army developed similar HCPs in 1955 and 1956, respectively (Henselman et al., 1995). Since their inception, military HCPs have served to increase awareness of the damaging effects of high-level noise exposure. They have also served to reduce the incidence and magnitude of NIHL in military personnel, primarily by mandating the use of personal protection devices (PPDs) such as sound-attenuating ear plugs or earmuffs in high-noise situations. However, NIHL remains a serious problem for military personnel who are exposed to loud noises during training and combat situations in which PPDs are either unavailable, impractical or dangerous to use, improperly fitted or worn, or inadequately designed to protect the ear from damage. As women become more fully integrated into all military occupational specialties, many will be placed at risk for developing NIHL. It is critical, therefore, that we understand the specific relationship between noise exposure and hearing loss in women, so that appropriate measures for preventing NIHL can be developed and implemented. Currently, very little is known about how women and men differ in their susceptibility to permanent hearing loss caused by exposure to continuous or impact/impulse noise.

Previous studies (e.g., Chung et al., 1983; Corso, 1963; Pearson et al., 1995; Ward, 1966) have reported small (generally less than 3 dB) differences between males and females in auditory sensitivity (i.e., thresholds for detecting pure tones under quiet listening conditions). In general, females tend to have slightly better pure-tone thresholds than males at frequencies above 1-2 kHz, while males may have slightly better thresholds below 1-2 kHz.. The reasons for the small but consistent gender differences are not clear. Some studies (Hellstrom, 1995a,b) suggest that gender differences in the acoustical properties of the external ear are responsible, and this is a reasonable hypothesis. However, some other gender differences, such as the greater incidence of spontaneous otoacoustic emissions among women than men (Bell, 1992; Bilger et al., 1990; Whitehead et al., 1989), cannot be accounted for in this manner, suggesting that factors other than simple acoustics may be involved. The first aim of our study is to examine sex differences in auditory sensitivity using the chinchilla, a species in which the female is slightly larger than the male (Clark, 1984).

Several studies have reported significant gender differences in temporary threshold shifts (TTS) caused by moderate-level noise exposures. Compared to males, females have been found to be less susceptible to TTS caused by low-frequency exposures, but *more* susceptible to TTS from high-frequency exposures (Petiot and Parrot, 1984; Ward, 1966). Currently, nothing is known about gender differences in permanent hearing loss induced by exposures to high-level, short-duration impulse noises that are more typical of military settings. Given the greater

susceptibility of females to high-frequency exposures and the broad-band spectral nature of short-duration impulse noises, however, it is reasonable to hypothesize that females may be more susceptible than males. A second aim of our project, therefore, is to investigate male/female differences in susceptibility to impulse noise, using an animal model where such differences are not confounded by differences in occupational and recreational noise exposure, diet, exercise, disease or exposure to ototoxic drugs.

A third aim of our research is to determine whether females and males differ in their ability to acquire resistance to NIHL through prophylactic "conditioning" exposures. Research conducted in the past few years has shown that the organ of Corti can be strengthened functionally, and perhaps physically, as a result of certain moderate-level exposures (e.g., Campo et al., 1991; Canlon et al., 1988; McFadden et al., 1996). That is, exposing an experimental subject to a moderate-level sound can render it more resistant to the damaging effects of a subsequent high-level exposure. The exposures that reduce susceptibility to acoustic trauma have been referred to as "conditioning" or "training" exposures (Canlon et al., 1988, 1995). Most previous investigations of the phenomenon have used conditioning exposures to provide protection from continuous high-level noise or tones. However, recent research conducted in our laboratory has shown that conditioning exposures can also provide protection from high-level impulse noise that produces direct mechanical damage to the cochlea. Specifically, Henselman et al. (1994) found that conditioning the auditory system of chinchillas with a low-frequency noise (0.5 kHz octave band noise at 95 dB SPL, 6 hr/day for 10 days) significantly reduced PTS caused by a subsequent exposure to 150 dB peak SPL impulse noise. The impulse noise used in Henselman's experiment was designed with a time-amplitude profile that simulated impulses created by a U.S. Army M-16A1 rifle. Other experiments conducted in our laboratory have demonstrated that the prophylactic effects of conditioning can persist for at least 30-60 days, implying that long-term physical or biochemical changes occur in the cochlea as a result of conditioning (McFadden et al., 1996).

The results of our conditioning experiments have exciting implications for preventing hearing loss in civilian employees and soldiers who are at risk for developing NIHL from exposure to high-level noise. In order for such an approach to be effective with both men and women in the military, however, it is first necessary to demonstrate that conditioning provides protection against hearing loss in both males and females, and then to determine the best conditioning exposures for producing protection.

This report describes research conducted during the period from September 23, 1996 to September 22, 1997. The initial experiments focused on sex differences in (a) auditory sensitivity prior to noise exposure, (b) susceptibility to temporary threshold shifts (TTS) and permanent threshold shifts (PTS) caused by exposure to high-level impulse noise (simulated M16 rifle fire), and (c) the ability to develop resistance to PTS through conditioning exposures (using low-frequency noise and UH-60 Blackhawk helicopter noise).

2. Body

2.1. Experimental Methods

All procedures described here were reviewed and approved by the University of Buffalo Animal Care and Use Committee, and conformed to NIH guidelines for the humane treatment of laboratory animals.

2.1.1. Subjects

A total of 64 chinchillas (*Chinchilla langier*; 32 female, 32 male) between 1 and 3 years of age were surgically prepared for the experiments described here. The chinchilla was selected for these studies because it (a) is relatively immune to middle ear infections and diseases that affect hearing (Clark, 1984); (b) has a relatively long life span (12-20 years) with minor age-related cochlear pathology and hearing loss (Bohne et al., 1990; McFadden et al., 1997a), so that results are not confounded by age-related changes occurring over the course of our experiments; (c) reacts predictably to anesthesia and tolerates surgery well, and (d) has a large auditory bulla that provides convenient access to the cochlea for histology. Most importantly, the chinchilla has a range of hearing that is more similar to that of humans than most other laboratory animals, particularly in the low frequencies (Miller, 1970; Heffner and Heffner, 1991), which enhances its suitability as a model for studying NIHL (McFadden et al., submitted). Because the chinchilla has been used for noise research for over 20 years, much is already known about its susceptibility to noise, and laboratory norms and procedures have been established using this species.

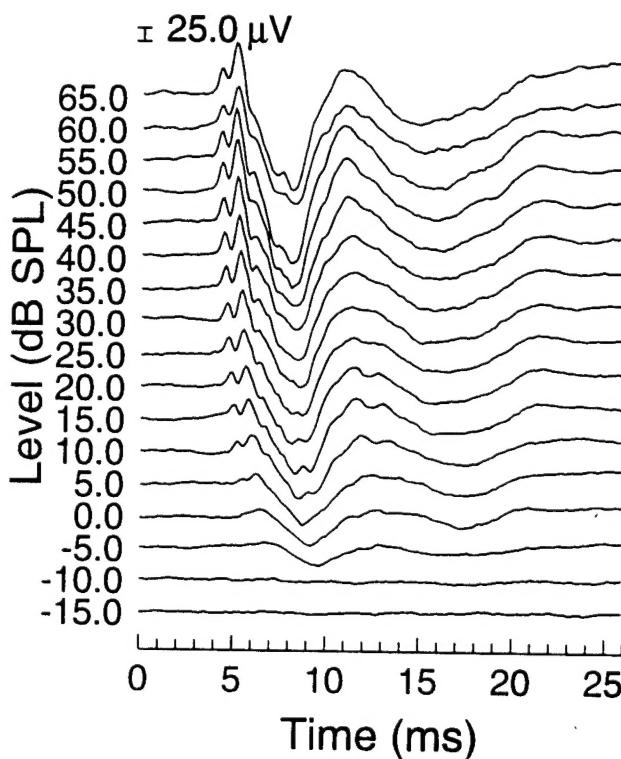
2.1.2. Surgical preparation

Each animal was deeply anesthetized with an intramuscular (i.m.) injection of ketamine hydrochloride (36 mg/kg) and acepromazine (0.56 mg/kg). Chronic recording electrodes were implanted in the right and/or left inferior colliculus (IC), and in the rostral cranium (McFadden et al., 1997a). Thirteen animals were implanted unilaterally; 42 animals were implanted bilaterally. A small hole was drilled in the dorsal cranium overlying the IC, and a recording electrode mounted on a stereotaxic device was advanced through the IC while the surgeon monitored sound-evoked electrical activity on audio and video monitors. When the electrode had been advanced to a depth that produced clear, large amplitude auditory evoked potentials (IC-EVPs), it was cemented to the skull with cyanoacrylic adhesive and dental cement. A short electrode was implanted in the rostral cranium to serve as the common lead for IC-EVP recording. Because the electrodes remain fixed in position, variability associated with changes in electrode placement between tests is eliminated. In addition, the signal-to-noise ratio is much better with implanted electrodes than with more conventional scalp electrodes, so that thresholds can be determined with greater precision. IC-EVPs recorded from electrodes implanted in this manner yield thresholds that are very close to behavioral thresholds measured in the same animals (Henderson et al., 1983), and about 15-20 dB lower than threshold estimates obtained using subcutaneous electrodes in the same animals (Murphy and Themann, 1995). Following surgery, the animals recovered in a quiet animal colony for at least one week prior to testing.

2.1.3. Measures of auditory function

The auditory sensitivity of each animal was assessed by measuring IC-EVPs. Cubic ($2f_1-f_2$) distortion product otoacoustic emissions (CDPs) were also obtained from most animals. Both measures are sensitive to outer hair cell (OHC) damage (McFadden et al., submitted; Decker, 1992), and have been used extensively in our lab (e.g., McFadden et al., 1997a,b). Together, these measures provide reliable information regarding the effects of noise on auditory function. All testing was conducted in a sound-attenuating booth (Industrial Acoustics Corp. 400) lined with sound-absorbing foam panels. The awake chinchilla was placed in a custom-designed tube (Snyder and Salvi, 1994) that held its head at a constant orientation within the calibrated sound field.

Fig. 1. IC-EVPs obtained in response to 8 kHz stimuli.



Stimuli for IC-EVP testing consisted of 10 ms tones (5 ms Blackman rise/fall ramp, alternating phase) at octave intervals from 0.5 to 16 kHz, presented at a rate of 20/sec. Stimuli were generated digitally (93 kHz sampling rate) by a 16 bit D/A converter on a digital signal processing (DSP) board (TMS320C25) in a personal computer (PC), and routed through computer-controlled attenuators and impedance matching transformers to a loudspeaker (Realistic 401197) located on the side of the test ear, at a distance of approximately 9 cm from the animal's pinna. The opposite (non-test) ear was plugged with a foam insert earplug, providing approximately 20-30 dB attenuation in addition to the attenuation produced by the

animal's head and body obstructing the propagation of sound to the opposite ear. Electrical activity from the IC electrode contralateral to the test ear was amplified (20,000 X), filtered (10-3000 Hz), and converted to digital signals (50 kHz sampling rate) by an A/D converter on a separate computer DSP board. Stimuli were presented in ascending order of frequency and intensity. Fifty or 100 trials were computer averaged at each stimulus level and the level was incremented in 5 dB steps. Figure 1 illustrates IC-EVP waveforms (raw data) obtained from a normal chinchilla.

Stored waveforms were analyzed visually to determine thresholds. Threshold (dB re: 20 µPa) was defined as the mid-point between the level at which there was a clear deflection in the

waveform and the next lower level at which there was none. For example, if there was a clear response at -5 dB and none at -10 dB, the threshold was recorded as -7.5 dB (see Fig. 1).

CDP measurements were made using a system designed in our lab that utilizes three DSP boards housed in a PC, insert earphones (Etymotic ER-2), a low noise probe microphone (Etymotic ER-10B), and custom-built attenuators and amplifiers. One DSP board processes microphone output while the other two generate digital signals (primary tones, f_1 and f_2). The primary tones were generated at a sampling rate of 93 kHz and output through 16 bit D/A converters. The microphone output was routed to a 16 bit A/D converter and digitized at a rate of 31 kHz. A Blackman windowing function was applied to the incoming data stream, and a partial discrete Fourier transform was computed. Frequency components corresponding to the two primary frequencies, the noise floor (f_n) and the cubic distortion product ($2f_1-f_2$) were computed. A calibration measurement preceded each input/output (I/O) function, in which the primary tones were presented at an attenuation of 20 dB and the output levels at the primary frequencies were measured and used as reference levels. Input/output functions were collected for primary tones ($f_2 = 1.2, 2.4, 3.6, 4.8, 7.2, 9.6$, and 12 kHz; $f_2/f_1=1.2$) from 0 to 70-80 dB SPL in 5 dB steps. CDP tests followed IC-EVP testing.

2.1.4. Noise exposures and acoustic calibration

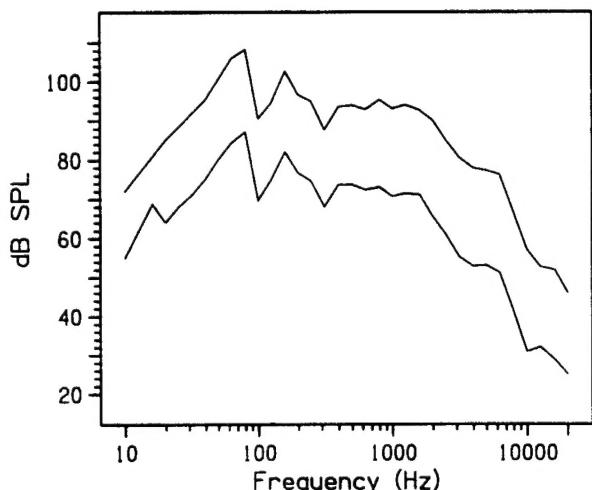
Noise stimuli used for conditioning exposures consisted of (a) octave band noise (OBN) centered at 0.5 kHz (McFadden et al., 1996), or (b) UH-60 helicopter noise, digitized from a cassette recording made from the cabin of a U.S. Army UH-60 Blackhawk helicopter while the aircraft was cruising at a speed of 120 knots (Henselman, 1995). The impulse noise, used for all animals, was a modified Friedlander wave (0.8 ms A-duration), with a time-amplitude profile simulating impulses created by 5.52 caliber rounds fired from a U.S. Army M16A1 rifle (Henselman et al., 1994).

Stimuli used for conditioning exposures were digitized with a 16-bit D/A converter on a DSP board (TMS320C25) in a PC and low-pass filtered (TDK HAF0030 active filter set at 20 kHz). The 0.5 kHz OBN was routed through a manual attenuator (Hewlett Packard 350D) to a low-distortion amplifier (NAD 2200), and delivered by a compression driver (JBL 2446J) fitted to a bi-radial exponential horn (JBL 2360H), suspended from the ceiling of the sound booth. The UH-60 helicopter noise was routed from the PC to a graphic equalizer (Technics SH 8065). Output from the graphic equalizer was routed to two band-pass filters (Krohn-Hite 3550), one set for 10-2000 Hz, the other for 1000-10,000 Hz. Output from each band-pass filter was amplified by separate amplifiers (NAD 2200) and attenuated by separate attenuators (HP 350D). The low-frequency portion of the UH-60 helicopter noise was delivered to a woofer (Power Logic HT 615), and the high-frequency portion was delivered to the compression driver (JBL 2446) and horn (JBL 2360).

For acoustic calibration of the conditioning noise, sound pressure levels (SPL re: 20 μ Pa) were measured with a calibrated Type I precision sound level meter (Larson-Davis 800B) and a 1/2" condenser microphone positioned at a height corresponding to the level of the ear canal of a standing chinchilla. SPL measurements were averaged across 5 positions within each cage (geometric center and each corner). Attenuator settings and cage positions were adjusted so that the average SPL in each cage was within 1 dB of the specified SPL. The spectra of the

conditioning noises were determined from 1/3-octave band level measurements made from 10 Hz to 20 kHz. Figure 2 shows the measured spectra for 90 dB SPL (lower curve) and 112 dB SPL (upper curve) UH-60 helicopter noise.

Fig. 2. Measured spectra of UH-60 helicopter noise at 90 dB SPL (lower curve) and 112 dB SPL (upper curve).



The impulse noise had a time-amplitude profile similar to that created by an M16 rifle (Henselman et al., 1994). The digital signal was converted to analog by a D/A converter on a DSP board, attenuated (HP 350D), amplified (NAD 2200), and routed in parallel to two compression drivers (JBL 2446) coupled to sound delivery tubes (5 cm dia X 20 cm). The ends of the sound delivery tubes were cut at 45° angles to broaden the spectral peak, thereby eliminating single-frequency resonance. The drivers faced each other, with the sound delivery tubes separated by 10 cm. Acoustic foam wedges surrounded the drivers to minimize reverberation. An animal was placed in a restraint tube in the 10 cm space between the opposing sound tubes. Fifty pairs of impulses, spaced

approximately 1000 ms apart (Henselman et al., 1994), were delivered simultaneously to both ears of the animal. The duration of the impulse exposure was approximately 1 min.

For impulse noise calibration, a 1/8" microphone (Brüel and Kjaer Model 4138) was placed at the position of the ear canal of a restrained animal. The voltage corresponding to a 114 dB, 250 Hz tone produced by a pistonphone coupled to the microphone was determined, and used to calculate the desired voltage for a 150 dB peak SPL signal. The attenuation was adjusted to produce the desired voltage.

All exposures were conducted in a 1.8 m X 2.0 m sound booth (Acoustic Systems), where animals were exposed singly (impulse noise) or in groups of 3-4 at a time (OBN and UH-60 helicopter noise). During exposure to conditioning noise, animals were housed in separate cages (approximately 27 cm X 21 cm X 22 cm) placed beneath the loudspeaker, and provided free access to food and water. Animals were rotated to different cages each day to minimize any effects of slight differences in SPL between cages.

2.1.5. Experimental groups and test schedule

Experimental animals were assigned to one of three "conditioning" groups: (1) Group 1, exposed to 90-95 dB 0.5 kHz octave band noise (OBN) for 6 hr/day for 5 consecutive days; (2) Group 2, exposed to 112 dB UH60 helicopter noise for 1.5 hr/day for 10 consecutive days; and (3) Group 3, exposed to 90 dB UH60 helicopter noise for 1.5 hr/day for 5 consecutive days. The animals were allowed to recover for 5 days prior to high-level noise exposure. After high-level exposure, animals were housed in a quiet animal colony for 25-35 days. A separate group of

animals (Group 4; Controls) was exposed to the high-level noise only. The groups and the numbers completing the entire experimental protocol in each group are shown in Table 1.

TABLE 1: Groups and Number of Ears Tested During Year 1.

GP	CONDITIONING STIMULUS	HIGH-LEVEL STIMULUS	# EARS TESTED	
			F	M
1	0.5 kHz OBN, 90-95 dB, 6 h/d, 5d	150 dB M16 rifle fire	7	8
2	UH-60 helicopter noise, 112 dB, 1.5 h/d, 10d	150 dB M16 rifle fire	7	9
3	UH-60 helicopter noise, 90 dB, 1.5 h/d, 5d	150 dB M16 rifle fire	12	12
4	NONE (CONTROL GP)	150 dB M16 rifle fire	15	13
			TOTAL	41 42

IC-EVPs and CDPs were recorded (a) prior to noise exposure ("baseline tests") in order to establish pre-exposure baselines, (b) at the end of the exposure period on Days 1, 5, and 10 of conditioning ("conditioning tests") in order to monitor TTS caused by the conditioning noise, (c) 5 days after conditioning to document recovery from TTS, (d) 15 min, 24 hr, and 5 days after high-level exposure ("post-exposure tests") in order to monitor TTS, and (e) after 25-35 days recovery from high-level exposure ("PTS tests") in order to determine permanent loss of auditory sensitivity. Prior to exposure, each animal was tested three times, and the average of the three measurements was used as the stable baseline estimate of sensitivity. Threshold shifts of each animal were calculated by subtracting mean pre-exposure IC-EVP thresholds from post-exposure thresholds. After 25-35 days recovery from high-level exposure, IC-EVPs and CDPs were measured on three separate occasions and averaged in order to calculate permanent threshold shifts at each frequency.

2.1.6. Data analyses

Data analyses were geared toward answering the following questions: (1) Are there significant sex differences in auditory sensitivity, IC-EVP amplitudes, or CDP I/O functions prior to noise exposure? (2) Are there sex differences in TTS and/or PTS caused by exposure to simulated M16 rifle fire? (3) Do female and male chinchillas differ in their responses to conditioning noises? (4) Do female and male chinchillas derive equivalent benefit (if any) from conditioning exposures, in terms of protection from PTS caused by high-level exposure? IC-EVP data from 83 ears of 55 animals were analyzed. Data from 9 additional animals were excluded from analysis due to high pre-exposure thresholds (n=2), technical problems (n=2), poor health (n=2), premature death (n=2), or electrode failure (n=1).

Analyses of variance (ANOVAs) were used to assess differences between means. The dependent variables (DVs) were IC-EVP thresholds and IC-EVP threshold shifts at various times

after noise exposure. Independent variables were Sex (a between-subjects factor), Frequency and Time of Assessment (within-subjects factors). Significant main effects and interactions involving Sex were analyzed further using one-way ANOVAs or t-tests. Within a group, changes as a function of time or frequency were assessed using paired t-tests. Mean IC-EVP and CDP amplitude functions for females and males were compared by calculating the 95% confidence interval for the difference between the means. All statistical tests were evaluated using a 0.05 criterion of significance.

2.2. Results and Discussion

We have made substantial progress toward completing the five experiments outlined for the first two years (Phase I) of the project. Three experiments were performed during Year 1, focusing on sex differences in: (1) auditory sensitivity prior to noise exposure, (2) susceptibility to hearing loss caused by high-level impulse noise exposure, and (3) the ability to benefit from conditioning exposures, in terms of reduced PTS from high-level noise exposure. Male and female chinchillas were conditioned with either UH-60 helicopter noise or 0.5 kHz OBN. Experimental (conditioned) and control animals were then exposed to simulated M16 rifle fire (150 dB peak SPL impulses). The inclusion of UH-60 helicopter noise and M16 rifle fire allowed us to examine sex differences in susceptibility to noises that soldiers might encounter during training or actual combat (Henselman, 1995).

IC-EVP data have been analyzed for four groups of animals (three conditioning groups plus one control group). Our preliminary results are exciting both in their parallels with the human data, and in their suggestion of sex differences in the ability to benefit from prophylactic conditioning exposures. The preliminary findings suggest some fundamental sex differences in the response of the cochlea to high-level impulse noise, and imply that human males and females may differ in their susceptibility to NIHL from high-level noises found in some military settings.

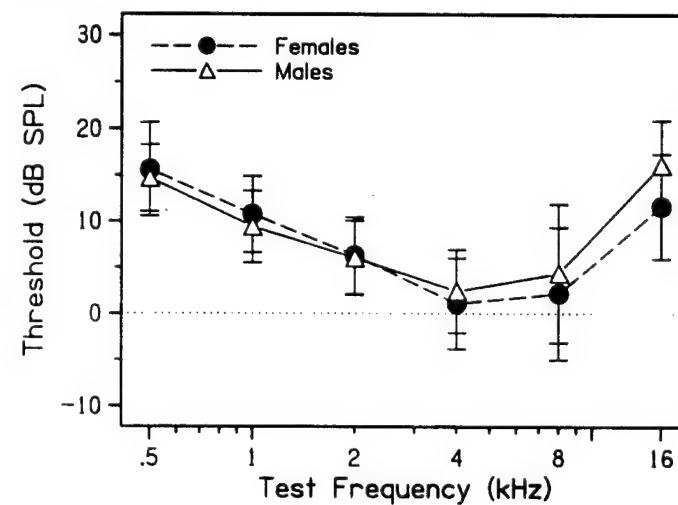
The results will be presented and discussed under three major headings, consistent with the technical objectives listed in the original project proposal: (1) Auditory sensitivity of females and males prior to noise exposure; (2) Sex differences in hearing loss from exposure to simulated M16 rifle fire; and (3) Sex differences in the ability to benefit from prophylactic conditioning exposures.

2.2.1. Auditory Sensitivity of Females and Males Prior to Noise Exposure

Small but consistent gender differences in auditory sensitivity have been well documented in humans (e.g., Chung et al., 1983; Corso, 1963; Pearson et al., 1995; Ward, 1966). In general, females tend to have slightly better (lower) pure-tone thresholds than males at frequencies above 1-2 kHz, while males may have slightly better thresholds below 1-2 kHz. Chung et al. (1983) analyzed data from more than 50,000 people and found that the average difference between males and females in hearing sensitivity was 2-3.5 dB for test frequencies above 2 kHz, and less than 1 dB for frequencies at or below 2 kHz. Ward (1966) found that thresholds of young adult females were up to 6 dB better than thresholds of young adult males at frequencies above 2.8 kHz.

Recently, Pearson et al. (1995) reported the results of the Baltimore Longitudinal Study of Aging, which tracked thresholds of 681 men and 416 women in low-noise occupations who were screened for otological disorders and noise-induced hearing loss. Their results provide further evidence of small gender differences in thresholds while ruling out occupational noise exposure as the cause for poorer thresholds in men. Women had significantly better thresholds than men at all frequencies above 1 kHz, while men had better thresholds at 0.5 kHz, and men and women did not differ at 1 kHz.

Fig. 3. Pre-exposure IC-EVP thresholds of female and male chinchillas.



Pre-exposure thresholds of a large group of chinchilla ears (55 female, 55 male) are shown in Figure 3. The pattern of sex differences observed in the chinchilla closely parallels the pattern described for humans. Male chinchillas have slightly lower thresholds than females at frequencies below 2 kHz, while female chinchillas have slightly lower thresholds than males at frequencies above 2 kHz. The differences are generally small, as they are for humans, but consistent. It is interesting to note that the largest divergence in mean thresholds occurs at the highest frequency tested (16 kHz). It is important to keep in mind that male and female chinchillas are raised under the same conditions, so that sex differences are not confounded by prior noise exposure history or by other factors that complicate the interpretation of human data.

A two-way mixed ANOVA, with Sex as a between-subjects factor and Frequency as a within-subjects factor, revealed a significant Sex X Frequency interaction, $F(5,540) = 7.58$, $p < 0.001$. Follow-up analyses indicated that mean thresholds at 16 kHz were significantly higher for males than for females (16.15 ± 4.8 dB vs. 11.64 ± 5.7 dB), $F(1,110) = 20.67$, $p < 0.0001$. Thresholds at frequencies below 16 kHz were not significantly different between the two sexes.

One factor that influences both auditory sensitivity and susceptibility to NIHL is the sound transfer function (STF), i.e., the way sound pressure is altered as sound passes from the

environment to the tympanic membrane (Hellstrom, 1995a,b). At low frequencies, the STF is primarily influenced by diffraction of sound around the torso, so STFs at lower frequencies depend primarily on the size and shape of the individual's body. At higher frequencies, the acoustic properties of the external ear (e.g., the length and cross-sectional area of the external auditory meatus, and the shape of the ear canal entrance) are the major determinants of the STF. Women tend to have shorter ear canals and smaller bodies than men. As a result, their STFs tend to be shifted to slightly higher frequencies compared to men (Hellstrom, 1995b). This fact could account for the slight, but consistent gender differences in human auditory thresholds. However, the similar sex differences seen in chinchillas are less likely to be due to acoustic factors, because female chinchillas tend to be slightly larger than male chinchillas. The results of our first experiment suggest that factors other than the acoustical properties of the external ear or the STF may be responsible for sex differences in auditory sensitivity.

Our results are consistent with other studies showing gender differences in human auditory function that cannot be accounted for by differences in STFs. For instance, gender differences have been observed in the upper limit for perceiving binaural beats (Tobias, 1965), with women having a significantly lower cut-off frequency than men (600 versus 800 Hz), in the incidence of spontaneous otoacoustic emissions, with women exhibiting them significantly more often than men (Bell, 1992; Bilger et al., 1990; Whitehead et al., 1989), and in auditory brainstem responses, with women having shorter central conduction times, even after differences in head size are taken into account (Patterson et al., 1981; Trune et al., 1978).

Mean IC-EVP input/output functions at 0.5, 1, 2, 4, 8 and 16 kHz are shown in Figure 4. The thin lines represent means for female chinchillas, and the hatched regions surrounding them represent the 95% confidence intervals. The thick lines represent the means for male chinchillas. It is apparent from Figure 4 that there were no significant sex differences in mean I/O functions prior to noise exposure, despite slight differences in IC-EVP thresholds (see Fig. 3).

Similarly, there were no meaningful differences between male and female chinchillas in their CDP I/O functions prior to noise exposure. Figure 5 shows average CDP I/O functions for a large group of ears. The thin lines represent means for females, and the hatched regions surrounding them represent the 95% confidence intervals. The thick lines represent means for males. The CDP frequency is indicated above each panel. CDP I/O functions were very similar for males and females, with thresholds around 20-30 dB SPL at all frequencies, and amplitudes increasing monotonically over the entire range of input levels. Overall, the results indicate that there are no meaningful sex differences in amplitudes of either IC-EVPs or CDPs prior to noise exposure.

Fig. 4. Mean pre-exposure IC-EVP input/output functions for females (thin lines) and males (thick lines). Hatched regions show 95% confidence intervals. Test frequency is indicated above each panel.

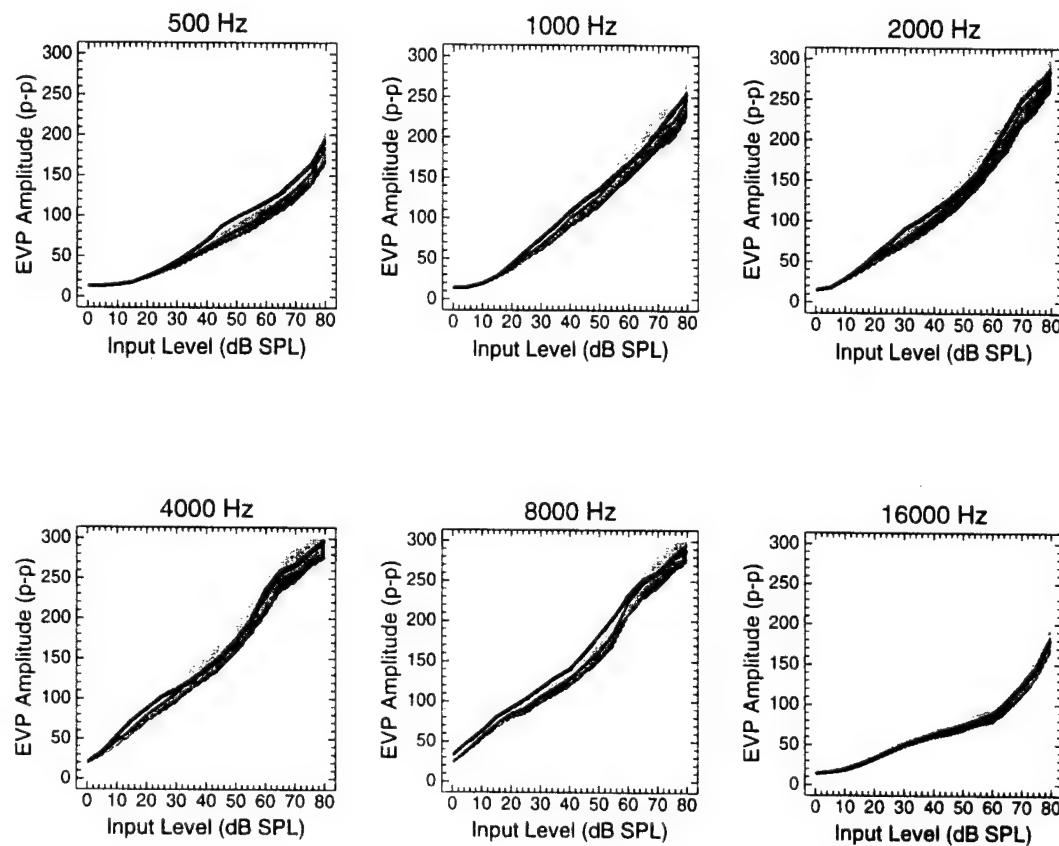
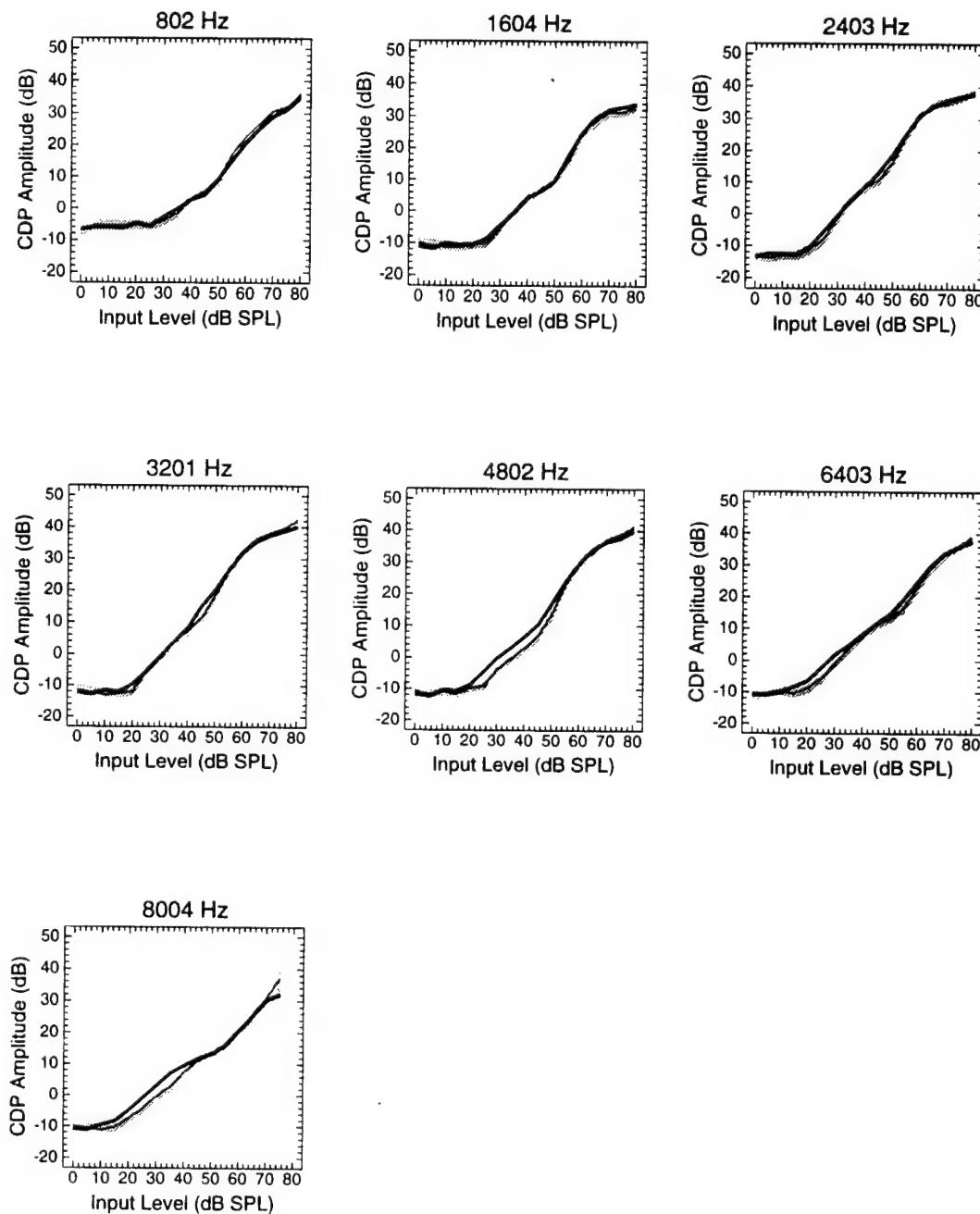


Fig. 5. Mean pre-exposure CDP input/output functions for females (thin lines) and males (thick lines). Hatched regions show 95% confidence intervals. CDP frequency is indicated above each panel.



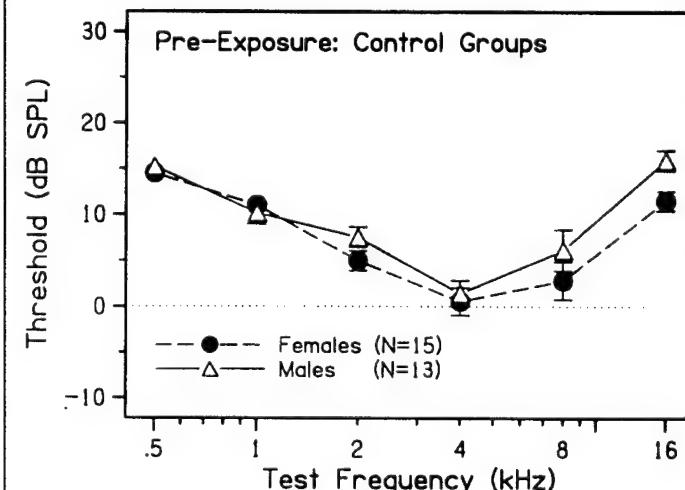
2.2.2. Sex Differences in Hearing Loss from Simulated M16 Rifle Fire

Gender differences have been reported in susceptibility to NIHL, both temporary (e.g., Axelsson and Lindgren, 1981; Dengerink et al., 1984; Petiot and Parrot, 1984; Ward, 1966) and permanent (e.g., Berger, Royster and Thomas, 1964; Gallo and Glorig, 1964). In general, experimental studies of TTS in humans have found that males exhibit more TTS than females from low-frequency exposures (below 2 kHz), whereas females exhibit more TTS than males from high-frequency exposures (above 2 kHz). In an early investigation of gender differences in susceptibility to TTS produced by high intensity tones and noise, Ward (1966) conducted 17 experiments with 24 male and 25 female adults. Females showed approximately 30% less TTS than males when the exposure frequency was below 1 kHz, but approximately 30% more TTS when the exposure frequency was above 2 kHz.

The above studies examined TTS rather than the more important issue of PTS simply because it is not ethical to intentionally induce PTS in human subjects. Most of what little we know about gender differences in PTS comes from retrospective studies of workers exposed to noise in industrial settings. Under these conditions, which typically involve exposure to low-frequency continuous noises, males typically develop much more hearing loss than females. Both Berger et al. (1964) and Gallo and Glorig (1964), for instance, found approximately 20 dB more PTS in males than in females after 9 years of industrial noise exposure. These results are consistent with the gender differences observed in Ward's (1966) studies of TTS. However, there are no comparable studies of gender differences in PTS produced by exposures to high-level impulse noises that are more typical of military exposures.

Our results from Control Group animals provide a perspective on sex differences in susceptibility to NIHL from high-level impulse noise. Pre-exposure IC-EVP thresholds for animals in the Control Group are shown in Figure 6. Although females exhibited slightly lower thresholds than males at 8 and 16 kHz, a two-way (Sex X Frequency) mixed ANOVA did not detect significant differences between the sexes. Thus, differences in thresholds observed after noise exposure (described below) can be attributed to sex-related factors other than pre-existing threshold differences.

Fig. 6. Pre-exposure thresholds of 15 female and 13 male ears of chinchillas in the Control Group.

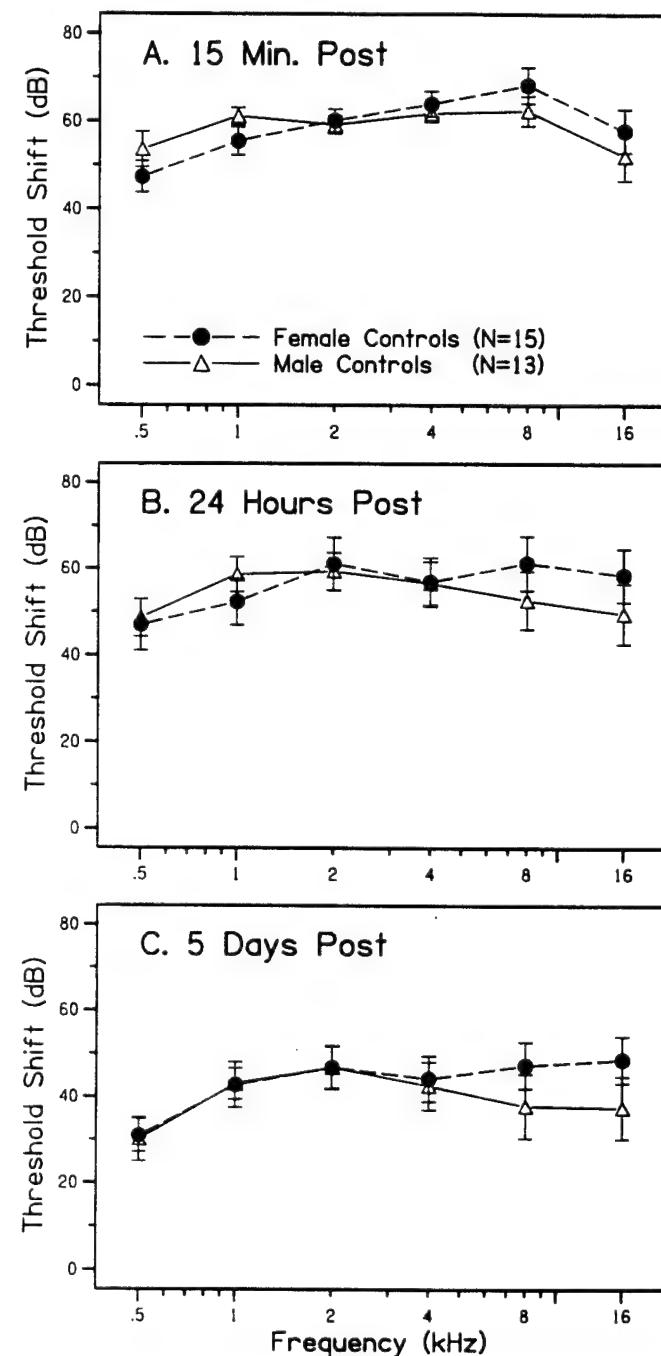


Mean IC-EVP threshold shifts measured at 15 min, 24 hr, and 5 days after exposure to 150 dB peak SPL impulse noise are shown in Figure 7.

When tested 15 min after the high-level exposure, both females and males exhibited significant threshold elevations (47-68 dB) at all frequencies (Fig. 7A). Males showed approximately 5-6 dB more TS than females at 0.5 and 1 kHz, whereas females showed approximately 6 dB more TS than males at 8 and 16 kHz. A two-way mixed (Sex X Frequency) ANOVA indicated that there was a significant Sex X Frequency interaction, $F(5,130)=2.1$, $p = 0.05$. Follow-up analyses indicated that TS increased progressively and significantly from 0.5 kHz to 4 kHz for females. TS was equivalent at 4 and 8 kHz, then declined significantly at 16 kHz. In contrast, males showed a much flatter pattern of TS, with equivalent TS at 1, 2, 4, and 8 kHz.

Relatively little threshold recovery occurred between 15 min and 24 hr post-exposure. Females showed TS decreases of 0-7 dB, while males showed TS decreases of 0-9 dB. Both females and males showed the greatest recovery (5-9 dB) at 4 and 8 kHz. As shown in Figure 7B, TS ranged from approximately 47 dB at 0.5 kHz to 61 dB at 8 kHz. Females exhibited approximately 9 dB more TS than males at 8 and 16 kHz, and approximately 5 dB less TS than males at 1 kHz. A two-way ANOVA yielded a significant Sex X Frequency interaction, $F(5,130)=2.4$, $p = 0.044$. Whereas females had equivalent TS at 2, 4, 8 and 16 kHz and less TS at 0.5 and 1 kHz, males had equivalent TS at 1, 2, 4 and 8 kHz, and significantly less TS at 0.5 and 16 kHz.

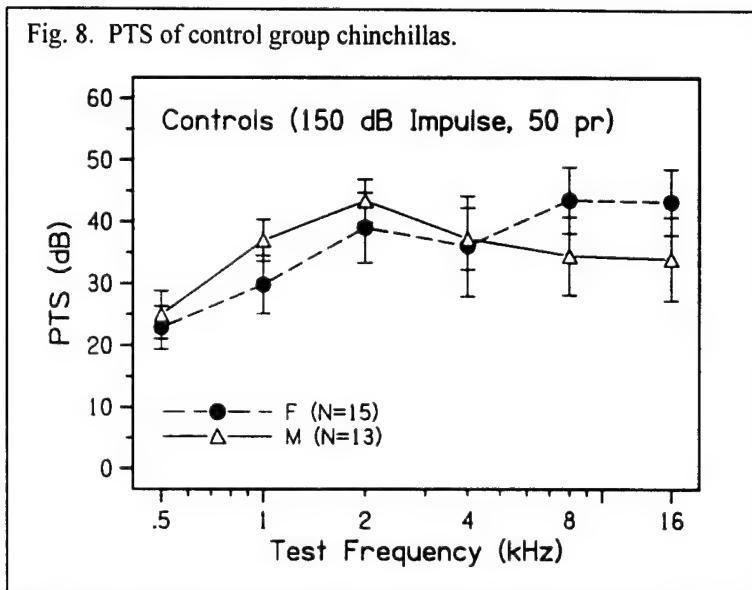
Fig. 7. Threshold shifts measured at various times after exposure to 150 dB peak SPL impulses.



Significant recovery occurred between 1 and 5 days after exposure, with TS decreasing by 9-19 dB. Females and males showed equivalent TS at frequencies below 4 kHz, whereas females exhibited approximately 9 dB and 11 dB more TS than males at 8 and 16 kHz, respectively (Fig. 7C). However, the two-way (Sex X Frequency) ANOVA did not reveal any significant differences between the sexes at this time.

Mean thresholds improved by 4-13 dB between 5 and 30 days post-exposure, when permanent hearing loss was assessed (Fig. 8). High-level exposure produced significant PTS at all frequencies for both females and males (paired t-tests; all p values < 0.001). PTS ranged from 23-43 dB, with females showing 2-7 dB less PTS than males at low frequencies (0.5-2 kHz), and approximately 9 dB more PTS at 8 and 16 kHz. A significant Sex X Frequency interaction was obtained, $F(5,130) = 3.10$, $p = 0.011$. Follow-up analyses indicated that both males and females developed

progressively and significantly greater PTS from 0.5 to 2 kHz. However, PTS peaked at 2 kHz for males, and declined significantly at higher frequencies. Females, in contrast, had significantly greater PTS at 8 and 16 kHz than at lower frequencies.



CDP amplitude data are generally consistent with the IC-EVP data. Before noise exposure, CDP I/O functions were similar for males and females, as shown in Figure 9. After exposure, however, CDP thresholds were elevated and amplitudes were significantly depressed. As shown in Figure 10, few sex differences were significant. However, there was a trend for males to have lower amplitude CDPs than females at low frequencies (where males had greater PTS), but higher CDP amplitudes at high frequencies (where males had less PTS).

The results from the control animals indicate that impulse noise simulating M16 rifle fire produces slightly more high-frequency hearing loss in female chinchillas than in males. The same noise produces slightly more low-frequency hearing loss in male chinchillas than in females. The results closely parallel results from human females and males in that females appear to be more susceptible to high-frequency hearing loss, while males appear to be more susceptible to low-frequency hearing loss from the same impulse noise exposure. The reasons for the sex differences in susceptibility to high-level impulse noise are unknown. As discussed previously, the STF of the external ear, which depends on physical characteristics such as the length and cross-sectional area of the ear canal and the shape of the ear canal entrance, is one factor that can influence susceptibility to NIHL (Hellstrom, 1995a). However, female chinchillas

Fig. 9. Mean pre-exposure CDP I/O functions for female (thin lines) and male (thick lines) controls.

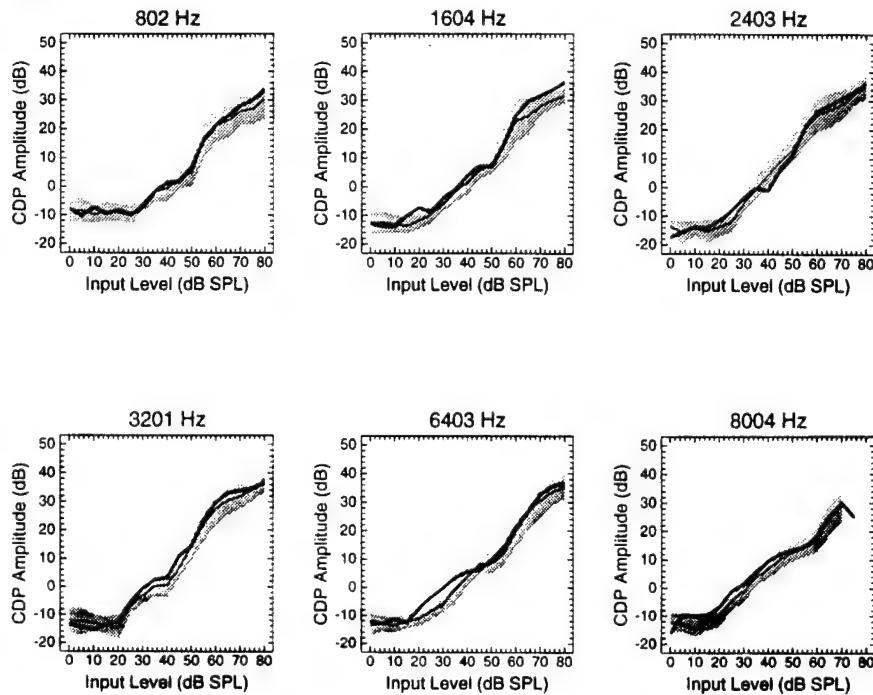
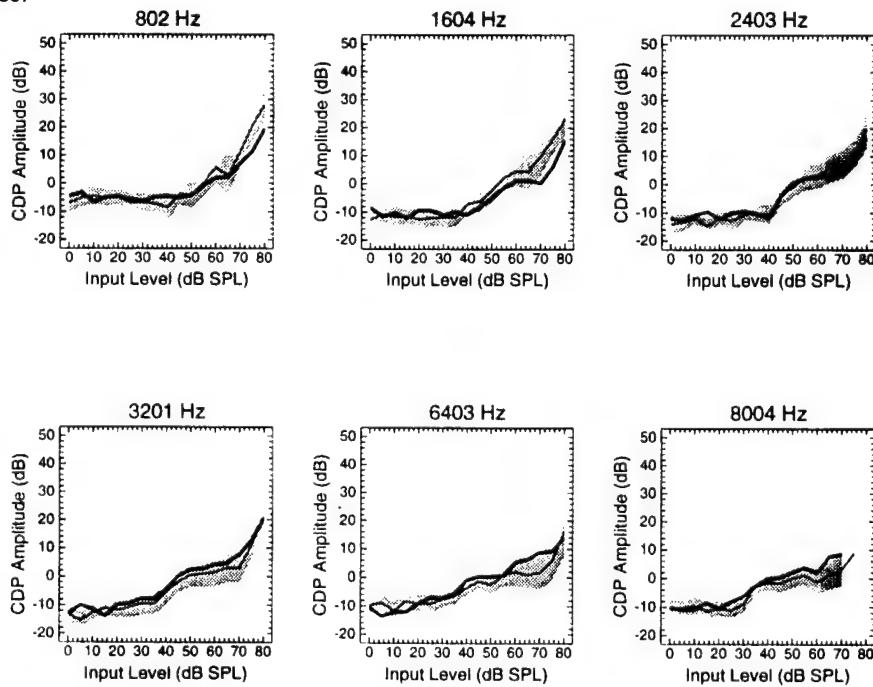
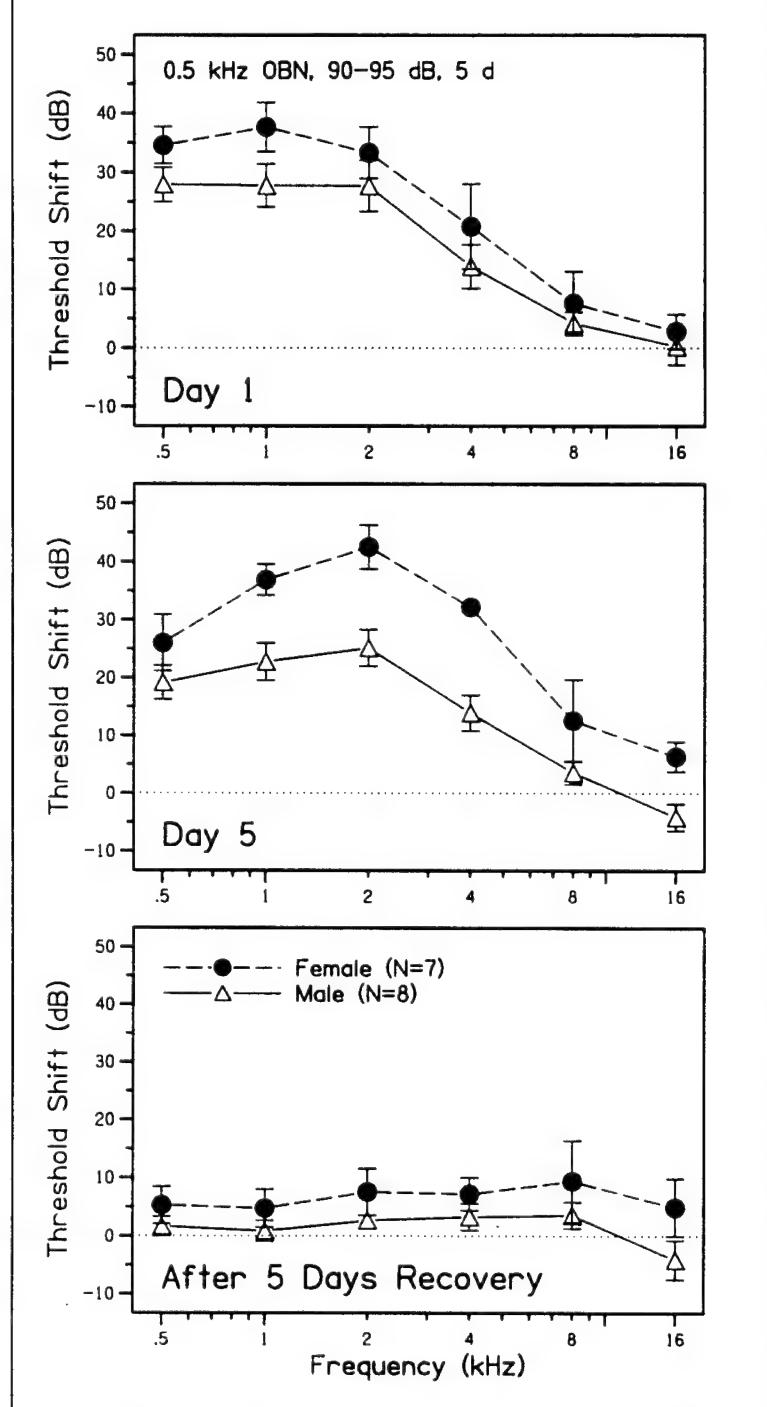


Fig. 10. Mean CDP I/O functions measure 30 days after exposure to 150 dB SPL impulse noise.



tend to be slightly larger than males, and there are no obvious differences in their pinnae or external ear canals. Thus, the results suggest that sex-related factors other than those related to body size or characteristics of the external ear may be important in determining susceptibility to high-level impact noise.

Fig. 11. Threshold shifts during and after a 5-day exposure to 0.5 kHz OBN conditioning noise (90-95 dB SPL).



2.2.3. Sex Differences in the Ability to Benefit from Conditioning Exposures

2.2.3.1. 0.5 kHz OB Noise

Pre-exposure thresholds of females and males assigned to Group 1 (0.5 kHz OBN conditioning group; see Table 1) were similar to those of control group animals (see Fig. 6). A two-way (Sex X Frequency) mixed ANOVA did not detect differences between males and females prior to exposure. Thus, differences in thresholds observed after noise exposure can be attributed to sex-related factors other than pre-existing threshold differences.

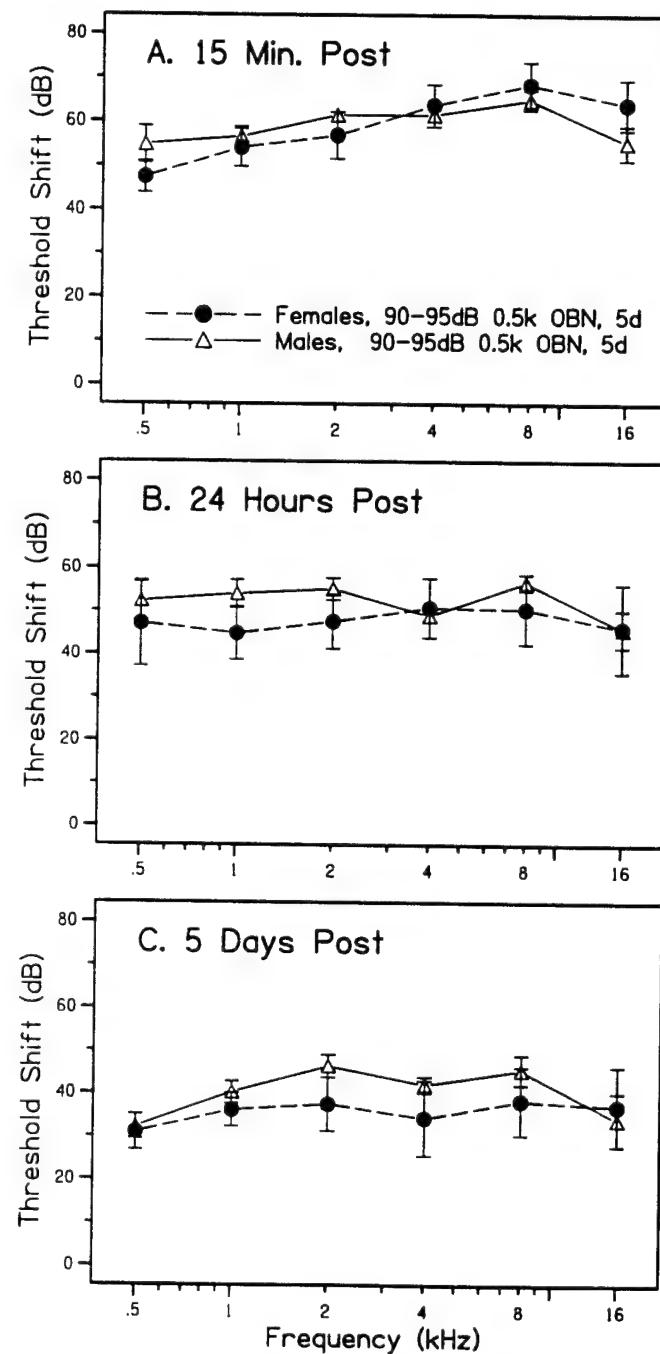
IC-EVP thresholds were measured after the first (Day 1) and last (Day 5) days of exposure to 90 dB OBN (6 hr/day), and again after five days of recovery from conditioning (Fig. 11). Conditioning noise produced significant TS at 0.5, 1, 2 and 4 kHz for both females and males on Days 1 and 5 (paired t-tests; all p values < 0.01). Females consistently showed more TS than males by 5-20 dB. Differences in TS between males and females were significant at 1, 2, 4 and 16 kHz on Day 5 of conditioning (Student t-tests; p values < 0.03).

After five days of recovery, thresholds recovered significantly at 0.5, 1, 2 and 4 kHz for both females (p values ≤ 0.001), and males (p values < 0.05). Thresholds measured 5 days after recovery were not significantly different from pre-exposure thresholds at any frequency for males. In contrast, females had significant residual TS at 2 kHz ($t(8) = -2.9$, $p = 0.02$) and 4 kHz ($t(8) = -3.5$, $p = 0.009$). Thus, females developed more TS during the 5-day exposure to 90-95 dB 0.5 kHz OBN exposure than males, and they recovered less completely over the subsequent 5 days than males.

After 5 days of recovery from 90-95 dB 0.5 kHz OBN noise exposure, Group 1 animals were exposed to 150 dB impulse noise. Mean TS measured at 15 min, 24 hr, and 5 days after the high-level exposure are shown in Figure 12.

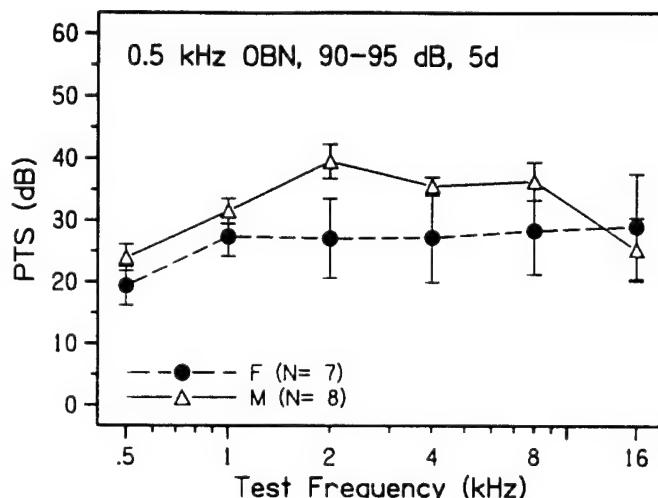
The pattern of TS seen in conditioned animals was very similar to that seen in non-conditioned control animals (Fig. 7). When tested 15 min after the high-level exposure, TS ranged from 47-68 dB (Fig. 12A), with females exhibiting slightly more TS than males at 8 and 16 kHz, and slightly less TS at 0.5, 1 and 2 kHz. Thresholds recovered by 0-18 dB over the first 24 hrs after exposure. More recovery occurred at high frequencies than at low frequencies, resulting in a relatively flat loss across frequencies. At 24 hrs post-exposure, females showed approximately 10 dB more recovery than males at 8 and 16 kHz.

Fig. 12. Threshold shifts of conditioned chinchillas after exposure to simulated M16 rifle fire.



Thresholds recovered by another 7-20 dB between 1 and 5 days post-exposure, and by 6-12 dB between 5 and 30 days post-exposure. Males exhibited more PTS than females at all frequencies below 16 kHz (Fig. 13). Sex X Frequency ANOVAs revealed no significant differences in TS between the sexes at any time post-exposure. However, this may have been due to insufficient statistical power to detect significant differences, given the small number ears in this group (7 female, 8 male).

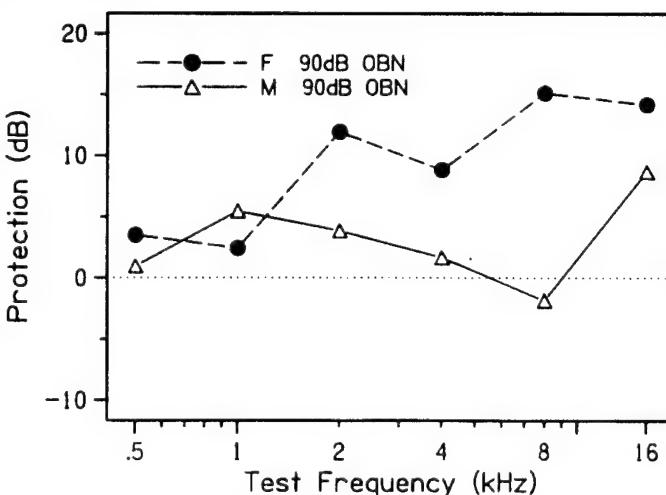
Fig. 13. PTS of chinchillas in Group 1.



The results from Group 1 suggest that females are more susceptible than males to hearing loss from a prolonged moderate-level exposure. The 5-day exposure to 90-95 dB low-frequency noise caused significantly greater TTS in females. However, the females were slightly more resistant to the damaging effects of a subsequent exposure to M16 rifle fire, showing up to 12 dB less PTS than males (Fig. 13). Females also showed greater benefit than males from conditioning. Figure 14 shows the differences in PTS between conditioned and control animals. The differences represent protection from PTS afforded by the conditioning exposures. Compared to females in the control group, females conditioned for 5 days with 0.5 kHz OBN showed 10-15 dB less PTS at all frequencies above 1 kHz. Males showed much less protection, with a maximum of 9 dB at 16 kHz. Thus, the 5-day conditioning protocol was more effective with females than males in providing protection from M16 rifle fire.

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Fig. 14. Protection from M16 rifle fire afforded by low-frequency conditioning.



2.2.3.2. UH-60 Helicopter Noise

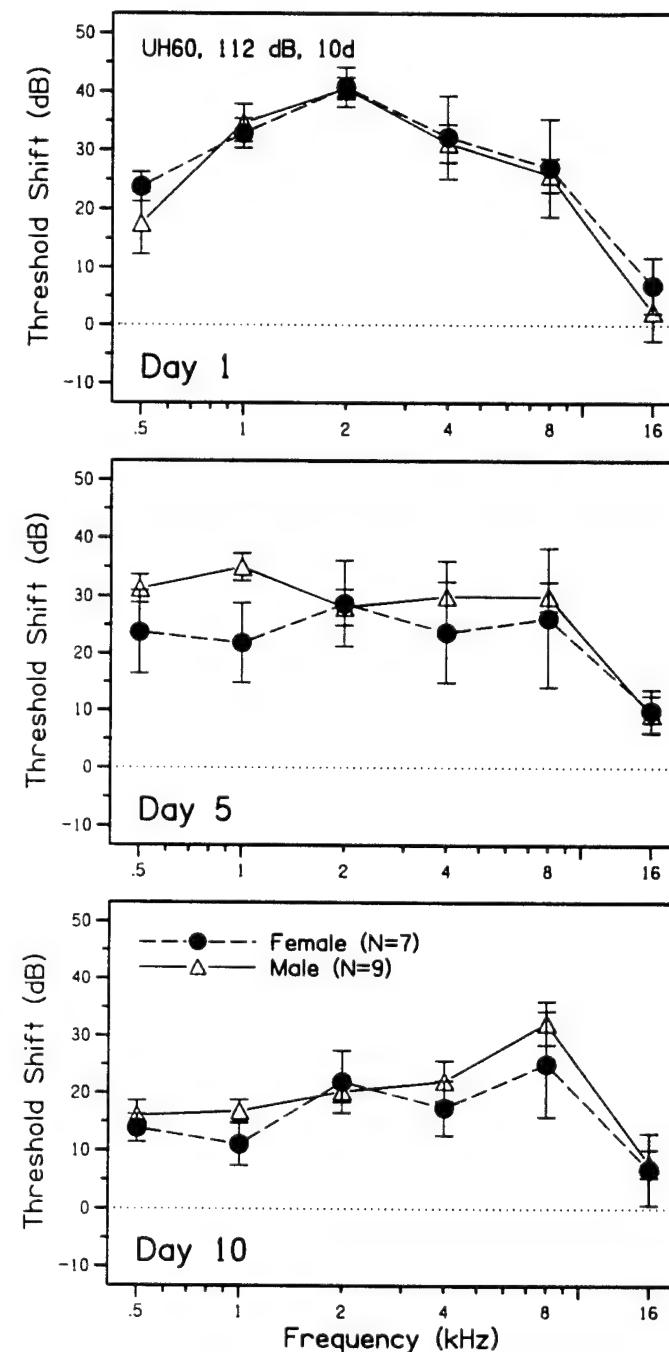
Pre-exposure thresholds of animals in Groups 2 and 3 were similar to those of animals in other groups (see Fig. 6). Animals in Group 2 were exposed to UH-60 Blackhawk helicopter noise at 112 dB SPL for 1.5 hr/day for 10 days. Animals in Group 3 were exposed to the helicopter noise at a lower level of 90 dB SPL, for 5 consecutive days.

Threshold shifts for Group 2 during conditioning are shown in Figure 15. After one day of exposure to 112 dB UH-60 noise, both females and males developed significant TS at all frequencies below 16 kHz, ranging from 17.5 dB at 0.5 kHz to 41 dB at 2 kHz. Males developed an additional 13 dB TS at 0.5 kHz between Day 1 and Day 5, but recovered by 13 dB at 2 kHz. Females showed similar recovery (12 dB) at 2 kHz. By the end of the tenth day of conditioning, thresholds below 8 kHz had recovered an additional 7-11 dB for females, and 8-18 dB for males. There were no significant differences between females and males at any time during conditioning.

Thresholds recovered by 7-18 dB for females, and by 11-33 dB for males during the 5-day recovery period. For males, there were no significant differences between pre-exposure thresholds and thresholds measured 5 days after conditioning. By contrast, thresholds of females remained significantly elevated at 0.5, 1, 4 and 16 kHz (paired t-tests, all p values < 0.05). Thus, females recovered less completely over the 5-day recovery period than males. This was also the case for conditioning with 0.5 kHz OBN, as described previously.

The pattern of TS after exposure to simulated M16 rifle fire was similar to that seen for control animals, with relatively flat TS from 1 to 8 kHz. TS of females ranged from 64-76 dB at 15 min, 56-66 dB at 24 hr, and 37-57 dB at 5 days post-exposure. TS of males ranged from 49-64 dB at 15 min, 43-54 dB at 24 hr, and 27-42 dB at 5 days. A two-way Sex X Frequency ANOVA indicated that females exhibited significantly more TS than males at 15 min post-exposure, $F(1,14)=9.00$, p =

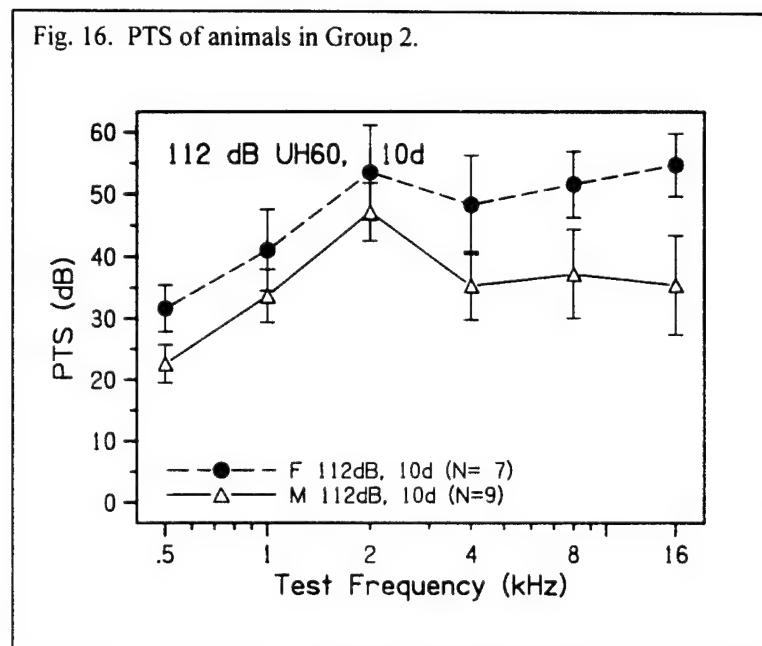
Fig. 15. Threshold shifts of Group 2 chinchillas during conditioning.



0.009. Follow-up analyses showed significantly greater TS for females at all frequencies above 0.5 kHz.

Figure 16 shows PTS of Group 2 animals. Thresholds were significantly elevated at all frequencies for both sexes (all p values < 0.003). Males showed peak PTS at 2 kHz, whereas females showed relatively flat PTS from 2 to 16 kHz. Females developed approximately 10-20 dB more PTS than males, with the greatest differences occurring at high frequencies. Thus, females appear to be more vulnerable than males to hearing loss from M16 rifle fire after a 10-day exposure to UH-60 helicopter noise, particularly at higher frequencies.

Fig. 16. PTS of animals in Group 2.



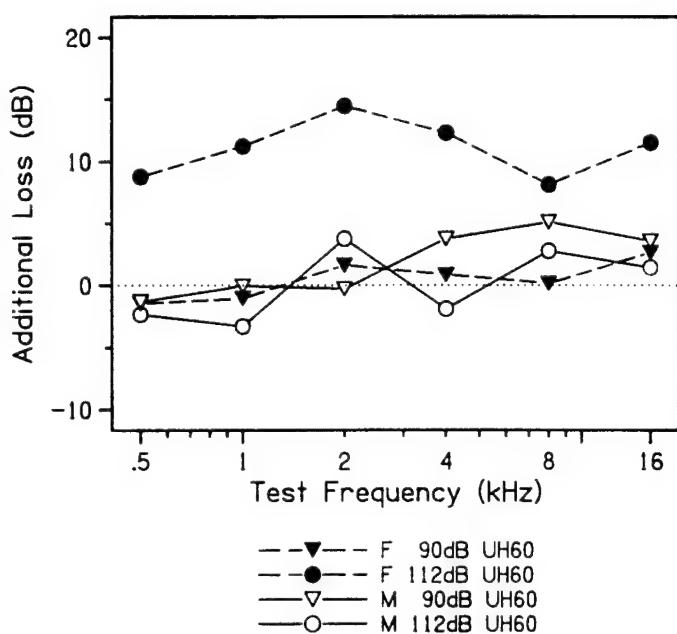
Group 3 animals showed very little TS during their 5 day conditioning exposure. On Day 1, thresholds were elevated by 10 dB or less, with the greatest shift at 2 kHz. Significant TS occurred at 2 kHz for females ($p < 0.001$), and at 1 and 2 kHz for males (p values < 0.01). On Day 5, thresholds were significantly elevated at 4 kHz as well, for both females ($p = 0.004$) and males ($p = 0.03$). Thresholds completely recovered by 5 days post-exposure for both sexes.

Exposure to M16 rifle fire produced patterns of TS and PTS that were very similar to those of control animals (see Figs. 7 and 8). As with control animals, females in Group 3 developed more PTS than males at 8 and 16 kHz, but less PTS than males at lower frequencies. As a result, there was a significant Sex X Frequency interaction, $F(5,110) = 2.45$, $p = 0.38$. As with control animals, both males and females developed progressively and significantly greater PTS from 0.5 to 2 kHz. Males showed a flat 40 dB loss from 2-16 kHz, whereas females had significantly greater PTS at 8 and 16 kHz than at lower frequencies except 2 kHz. These results indicate that a 5-day exposure to UH-60 helicopter noise produced no damage by itself, but it also produced no protection from subsequent exposure to M16 rifle fire.

Overall, the results from Groups 2 and 3 show that exposure to UH-60 helicopter noise produces no significant protection from M16 rifle fire. In fact, repeated exposure to high-level helicopter noise, as would occur if soldiers wore no hearing protectors when flying, increases susceptibility to damage from a subsequent high-level exposure, particularly for females. This is illustrated in Figure 17, which shows additional loss incurred by animals exposed to helicopter noise and M16 rifle fire, compared to control animals exposed only to M16 rifle fire. Animals exposed to 90 dB helicopter noise developed no additional losses. By contrast, animals exposed to 112 dB helicopter noise developed additional losses ranging from 2-15 dB. Males developed additional losses at high frequencies, whereas females developed an average of 10 dB more loss

across all frequencies. These results suggest that failure to wear PPDs during prolonged exposure to low-frequency helicopter noise may compromise the ear's ability to cope with a subsequent high-level exposure.

Fig. 17. PTS of animals in Groups 2 and 3 compared to PTS of like-sexed control animals.



2.3. Recommendations

In the original proposal for this project, the following questions were listed as targets of study during Years 1 and 2 (Phase I of the project):

1. Are there consistent differences between male and female chinchillas in hearing sensitivity?
2. How do female chinchillas compare to males in their susceptibility to NIHL caused by impulse noise typical of noises found in military environments?
3. Do conditioning exposures produce equivalent protection for male and female chinchillas against damage and hearing loss caused by subsequent high-level noise exposure?

The experiments conducted in Year 1 have begun to answer these important questions. In addition, we have met most of the technical objectives and completed most specific tasks listed for Year 1. In the following section, our original technical objectives for Year 1 are presented in italicized text. Statements regarding our progress toward accomplishing the objectives are presented in regular text.

Technical Objective #1: Examine auditory sensitivity before, during and after noise exposure as a function of sex (male versus normal female chinchillas) and conditioning exposure.

[NOTE: The complete protocol, from surgical preparation to final testing, will require approximately 2 months for each group of 4-6 animals (see Fig. 7). Animals will be prepared, tested, and exposed to noise in groups of 4-6 at a time. Some overlap in exposures and recovery occurs, so that Phase I of the project will require approximately 22 months for all exposures and data collection to be completed.]

Task 1: Months 1-6: Surgically prepare first 20 male and 20 female chinchillas for auditory evoked potential (AEP) recordings. [Note: Approximately 4 animals can be surgically prepared in a week. However, because space in the animal colony is limited and the noise exposure booth

must be scheduled for use, there may be some weeks when no new animals can be introduced into the experiment.]

Months 8-14: Surgically prepare 40 more chinchillas.

Sixty-four chinchillas were surgically prepared for IC-EVP testing during the past year. The next group of 10 animals is scheduled for surgery in late October. We are hoping to complete Phase I of the project with approximately 10 fewer animals than originally estimated, primarily due to an exceptionally high survival and completion rate during Year 1 (only 9 of the surgically prepared chinchillas failed to complete the entire experimental protocol, leaving us with data from 55 animals).

Task 2: *Months 1-18: Perform preliminary hearing tests (3 AEP and 3 CDP tests) on surgically prepared animals.*

Pre-exposure IC-EVPs were obtained from a total of 55 animals. Most animals were also tested for CDPs. Each animal was tested three times, and the measurements were averaged for stable baseline estimates of IC-EVP thresholds and CDP input/output functions.

Task 3: *Months 1-7: Expose 10 males and 10 females to 10 days of conditioning noise followed 5 days later by exposure to impulse noise. Test hearing during and after exposures (i.e., on Days 1, 5, and 10 of conditioning, 5 days after conditioning, and at 15 min., 24 hr, and 10 days after high-level exposure). Expose 10 male and 10 female control animals to impulse noise alone; test at 15 min, 24 hr, and 5 days post-exposure.*

All animals will be tested three more times after 30 days of recovery from high-level noise, then their cochleas will be collected for histological analysis.

Months 9-22: Expose animals to appropriate conditioning and high-level noises; test hearing during and after exposure.

Data collection is essentially complete for the 10-day 112 dB UH-60 helicopter noise group and 100% complete for the control group. Female and male chinchillas in the UH-60 noise group were exposed for 1.5 hr/day for 10 days. Hearing tests were performed on Days 1 and 10 of conditioning and 5 days after conditioning. Conditioned and control animals were exposed to M16 rifle fire, and tested at 15 min, 24 hr, 5 days, and 25-35 days after high-level exposure. Cochleas were collected for histological analyses, which will be performed during Year 2.

Our current procedure of implanting IC recording electrodes bilaterally permits us to test both ears of an animal. Data were obtained from 7 and 9 ears of female and male chinchillas, respectively, in the UH-60 noise group, and from 15 and 13 ears of female and male chinchillas in the control group. We believe that sufficient data have been obtained to draw reliable conclusions about sex differences in susceptibility to prolonged exposure to UH-60 helicopter noise and to M16 rifle fire (control group).

Task 4: *Months 1-22: On-going data entry and analysis.*

Data collection will be completed for the 10-day helicopter noise conditioning group and the impulse noise control group by Month 11.

Data collection for the two 5-day helicopter noise conditioning groups will be completed by Month 19.

Data collection for the low-frequency conditioning noise group will be completed by Month 22.

Data collection has been completed for the 10-day helicopter noise conditioning group and the impulse noise control group. Data collection is approximately 90% complete for the low-frequency conditioning noise group. We will continue to collect data from animals in 5-day UH-60 conditioning groups during Year 2, and do not anticipate any problems completing data collection by Month 22, as originally planned.

Task 5: *Month 11: Preparation of data for presentation at professional meeting, describing sex differences in the response of the auditory system to high-level impulse noise.*

A manuscript describing sex differences in auditory sensitivity and susceptibility to noise is currently in preparation. In addition, the PI will discuss the data with professionals in the field of hearing science, audiology and otolaryngology at the upcoming meeting of the Association for Research in Otolaryngology (scheduled for February, 1998).

Task 6: *Month 12: Prepare detailed annual report to summarize project findings and progress.*

The current report summarizes experiments performed during Year 1 of the project, and current progress on completing Phase I of the project.

3. Conclusions

The purpose of Phase I of this project is to investigate sex differences in auditory sensitivity, susceptibility to NIHL caused by impact noise, and the ability to develop resistance to NIHL through "conditioning" exposures. Hearing function was assessed by measuring sound-evoked electrical activity from the inferior colliculus and distortion product otoacoustic emissions from the ear.

The results of our experiments in Year 1 can be summarized as follows:

1. Chinchillas exhibit small, but consistent sex differences in auditory sensitivity, with females showing slightly better thresholds at high frequencies, but slightly worse thresholds at low frequencies than males. This pattern matches the pattern described for humans.
2. Chinchillas exhibit sex differences in their susceptibility to hearing loss caused by exposure to high-level (150 dB peak SPL) impulse noise (simulated M16 rifle fire). For a given exposure, female chinchillas develop less hearing loss at low frequencies, but more hearing loss at high frequencies than males.
3. Chinchillas exhibit sex differences in hearing loss caused by moderate-level low-frequency noise (i.e., 90-95 dB SPL 0.5 kHz OBN). Females show more TTS during exposure and less complete recovery after exposure.

4. A 5-day 0.5 kHz OBN conditioning protocol reduces hearing loss from simulated M16 rifle fire for both female and male chinchillas. However, female chinchillas show relatively more benefit than males.
5. Exposure to UH-60 helicopter noise at 112 dB SPL enhances susceptibility to hearing loss caused by a subsequent exposure to simulated M16 rifle fire, with susceptibility increasing more for females than for males.
6. Exposure to UH-60 helicopter noise at 90 dB SPL (i.e., a level that might be experienced by a soldier wearing PPDs while riding in the cabin of the helicopter) neither increases nor decreases susceptibility to hearing loss caused by a subsequent exposure to simulated M16 rifle fire. There are no sex differences in this regard.

The results from the experiments described here have important practical and theoretical implications. From a theoretical standpoint, they provide a much-needed perspective on the role of sex-related factors on normal physiology and function, thereby increasing our understanding of basic auditory system physiology. From a practical standpoint, they can aid in designing programs and procedures for reducing hearing loss in military personnel who are exposed to traumatic noise. Overall, the data point to important sex differences in the response of the cochlea to high-level impulse noise and conditioning, which could have important implications for military assignments and hearing conservation programs.

4. References

- Axelsson, A. and Lindgren, F. (1981) Pop music and hearing. *Ear Hear* 2, 64-69.
- Bell, A. (1992) Circadian and menstrual rhythms in frequency variations of spontaneous otoacoustic emissions from human ears. *Hear Res* 58, 91-100.
- Berger, E.H., Royster, L.H. and Thomas, W.G. (1978) Presumed noise-induced permanent threshold shift resulting from exposure to an A-weighted Leq of 89 dB. *J Acoust Soc Am* 64, 192-197.
- Bilger, R.C., Matthies, M.L., Hammel, D.R. and DeMorest, M.E. (1990) Genetic implications of gender differences in the prevalence of spontaneous otoacoustic emissions. *J Sp Hear Res* 33, 418-432.
- Bohne, B.A., Gruner, M.M. and Harding, G.W. (1990) Morphological correlates of aging in the chinchilla cochlea. *Hear Res* 48, 79-91.
- Campo, P., Subramaniam, M. and Henderson, D. (1991) Effect of "conditioning" exposures on hearing loss from traumatic exposure. *Hear Res* 55, 195-200.
- Canlon, B., Borg, E. and Flock, A. (1988) Protection against noise trauma by preexposure to low level acoustic stimulus. *Hear Res* 34, 197-200.
- Canlon, B. and Dagli, S. (1995) Protection against temporary and permanent noise-induced hearing loss by sound conditioning. In: A. Axelsson et al. (Eds.) *Scientific Basis of Noise-Induced Hearing Loss*. New York: Thieme, pp. 172-180.
- Chung, D.Y., Mason, K., Gannon, R.P. and Willson, G.N. (1983) The ear effect as a function of age and hearing loss. *J Acoust Soc Am* 73, 1277-1282.
- Clark, J.D. (1984) Biology and diseases of other rodents. In: J.G. Fox, B.J. Cohen and F.M. Lowe (Eds.), *Laboratory Animal Medicine*. New York: Academic Press Inc.
- Corso, J.F. (1963) Age and sex differences in pure tone thresholds: survey of hearing levels from 18 to 65 years. *Archs Otolaryngol* 77, 385-405.
- Dancer, A. and Franke, R. (1986) Effects of weapon noise on hearing. In: R.J. Salvi, D. Henderson, R.P. Hamernik and V. Colletti (Eds.), *Basic and Applied Aspects of Noise-Induced Hearing Loss*. New York: Plenum Press.
- Decker, T.N. (1992) *Otoacoustic Emissions. Seminars in Hearing Vol. 13(1)*. New York: Thieme Medical Publishers, Inc.
- Dengerink, J.E., Dengerink, H.A., Swanson, S., Thompson, P. and Chermak, G.D. (1984) Gender and oral contraceptive effects on temporary auditory effects of noise. *Audiol* 23, 411-425.
- Gallo, R. and Glorig, A. (1964) Permanent threshold shift changes produced by noise exposure and aging. *J Ind Hyg* 25, 237-245.
- Heffner, R.S. and Heffner, H.E. (1991) Behavioral hearing range of the chinchilla. *Hear Res* 52, 13-16.
- Hellstrom, P.-A. (1995a) Individual differences in peripheral sound transfer function: Relation to NIHL. In: A. Axelsson et al. (Eds.), *Scientific Basis of Noise-Induced Hearing Loss*. New York: Thieme, pp. 110-116.
- Hellstrom, P.-A. (1995b) The relationship between sound transfer functions and hearing levels. *Hear Res* 88, 54-60.

- Henderson D, Campo P, Subramaniam M, Fiorino F. Development of resistance to noise. In: A. Dancer, D. Henderson, R.J. Salvi and R.P. Hamernik (Eds.), *Noise Induced Hearing Loss*. St. Louis: Mosby-Year Book.
- Henderson, D., Spongr, V.P., Subramaniam, M. and Campo, P. (1994) Anatomical effects of impact noise. *Hear Res* 76, 101-117.
- Henderson, D., Subramaniam, M. and Boettcher, F.A. (1993) Individual susceptibility to noise-induced hearing loss: An old topic revisited. *Ear Hear* 14, 152-168.
- Henderson, D., Hamernik, R.P. and Sitler, R.W. (1974) Audiometric and histological correlates of exposure to 1-ms noise impulses in the chinchilla. *J Acoust Soc Am* 56, 1210-1221.
- Henderson, D., Hamernik, R.P., Salvi, R.J. and Ahroon, W.H. (1983) Comparison of auditory-evoked potentials and behavioral thresholds in normal and noise-exposed chinchillas. *Audiol* 22, 172-180.
- Henselman, L.W. (1995) Effect of "conditioning" on hearing loss from military noise exposures. Unpublished Doctoral Dissertation, State University of New York at Buffalo.
- Henselman, L.W., Henderson, D., Shadoan, J., Subramaniam, M., Saunders, S. and Ohlin, D. (1995) Effects of noise exposure, race, and years of service on hearing in U.S. Army soldiers. *Ear Hear* 16, 382-391.
- Henselman, L.W., Henderson, D., Subramaniam, M. and Sallustio, V. (1994) The effect of 'conditioning' exposures on hearing loss from impulse noise. *Hear Res* 78, 1-10.
- McFadden, S.L., Campo, P., Quaranta, N. and Henderson, D. (1997a) Age-related decline of auditory function in the chinchilla (*Chinchilla laniger*). *Hear Res* 111, 114-126.
- McFadden, S.L., Quaranta, N. and Henderson, D. (1997b) Suprathreshold measures of auditory function in the aging chinchilla. *Hear Res* 111, 127-136.
- McFadden, S.L., Campo, P., Ding, D.L. and Quaranta, N. Effects of noise on inferior colliculus evoked potentials and cochlear anatomy in young and aged chinchillas. *Hear Res* (submitted).
- McFadden, S.L., Henderson, D. and Shen, Y.-H. (1996) Low-frequency "conditioning" provides long-term protection from noise-induced threshold shifts in chinchillas. *Hear Res* 103, 142-150.
- McFadden, S.L., Kasper, C., Ostrowski, J., Ding, D.L. and Salvi, R.J. Effects of inner hair cell loss on inferior colliculus evoked potential thresholds, amplitudes and forward masking functions in chinchillas. *Hear Res* (submitted).
- Miller, J.D. (1970) Audibility curve of the chinchilla. *J Acous Soc Am* 48, 513-523.
- Murphy, W.J. and Themann, C.L. (1995) Hearing thresholds from auditory evoked potentials: Subcutaneous and implanted electrodes. *Assoc Res Otolaryngol* 548.
- Pearson, J.D., Morrell, C.H., Gordon-Salant, S., Brant, L.J., Metter, E.J., Klein, L.L. and Fozard, J.L. (1995) Gender differences in a longitudinal study of age-associated hearing loss. *J Acoust Soc Am* 97, 1196-1205.
- Petiot, J.-C. and Parrot, J.E. (1984) Effects of the ovarian and contraceptive cycles on absolute thresholds auditory fatigue and recovery from temporary threshold shifts at 4 and 6 kHz. *Audiol* 23, 581-598.
- Snyder, D.L. and Salvi, R.J. (1994) A novel chinchilla restraint device. *Lab Anim* 23, 42-44.

- Tobias, J.V. (1965) Consistency of sex differences in binaural beat perception. *Int Audiol* 4, 179-182.
- Trune, D.R., Mitchell, C. and Phillips, D.S. (1978) The relative importance of head size, gender and age on the auditory brainstem response. *Hear Res* 32, 165-174.
- Ward, D.W. (1966) Temporary threshold shift in males and females. *J Acoust Soc Am* 40, 478-485.
- Whitehead, M.L., Baker, R.J. and Wilson, J.P. (1989) The bilateral symmetry and sex asymmetry of spontaneous otoacoustic emission (SOAE) incidence in human ears. *Br J Audiol* 23, 149.